

## SHORT COMMUNICATION

**Benzoquinone-rich exudates from the harvestman *Pachylus paessleri* (Opiliones: Gonyleptidae: Pachylinae)**

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**Abstract.** The chemical composition of the scent gland secretion of *Pachylus paessleri* Roewer 1913, a pachyline harvestman, was analysed by gas chromatography–mass spectrometry. The secretion is a six-component mixture of benzoquinones, with 2,3-dimethyl-1,4-benzoquinone and 2,3,5-trimethyl-1,4-benzoquinone being the main components (together amounting for ¾ of the secretion). Minor components are 2,5-dimethyl-1,4-benzoquinone (about 12%), 2-ethyl-3-methyl-1,4-benzoquinone (about 8%), 2,5-dimethyl-3-ethyl-1,4-benzoquinone (5%), and 2-ethyl-5-methyl-1,4-benzoquinone (about 1%). No sex-dependent differences could be detected. While dimethyl- and trimethyl-benzoquinones are widespread in scent gland secretions of Gonyleptoidea, 2,5-dimethyl-3-ethyl-1,4-benzoquinone and 2-ethyl-5-methyl-1,4-benzoquinone are reported for the first time in Opiliones. The phylogenetic implications of these compounds are briefly discussed in the scope of the present knowledge of Laniatores.

**Keywords:** Chemical ecology, defense, exocrine products, Arachnida, chemosystematics

A pair of large prosomal scent glands is an important synapomorphy of all Opiliones (Machado et al. 2007; Gnaspini & Hara 2007). These glands have traditionally been considered to play a role in chemical defense (e.g., Martens 1978) and have been the subject of several investigations since the 1950's. Chemical data from scent gland secretions, even though strongly biased regarding certain groups, are meanwhile available for all four currently recognized suborders. So far, the chemical knowledge on the Cyphophthalmi, Eupnoi and Dyspnoi appears to be rather poor, relying on only three species of Cyphophthalmi (Raspotnig et al. 2005; Jones et al. 2009), about one dozen species of Eupnoi (e.g., Ekpa et al. 1985) and only one representative of Dyspnoi (Raspotnig et al. 2010). Also within the Eupnoi, data are strongly biased, mainly being available for North American Sclerosomatidae, *Leiobunum* spp. and one *Hadrobunus* species (Ekpa et al. 1985) and for one single species of Phalangidae (Wiemer et al. 1978). In the Laniatores the situation appears to be similar: even though about 35 species have already been investigated, the major part of data originates from Gonyleptoidea such as cosmetids, gonyleptids, and manaosbiids (see Hara et al. 2005 and Gnaspini & Hara 2007 for references). In addition, chemical data are available for one species of Stygnomatidae (Duffield et al. 1981), one species of Travuniidae (Ekpa et al. 1984) and recently also for two species of Phalangodidae (Shear et al. 2010a). Chemical profiles of scent gland secretions in Opiliones are generally assumed to bear phylogenetically important information, as already emphasized by several authors (e.g., Duffield et al. 1981; Roach et al. 1980). In Gonyleptoidea, scent gland secretion bouquets are characterized by seemingly species-specific combinations of alkylated benzoquinones and phenols. Initial attempts to map these chemical products onto a preliminary gonyleptid phylogeny have already been published (Hara et al. 2005).

As outlined above, the use of scent gland profiles in opilionid systematics generally suffers from a hitherto rather poorly-founded chemical matrix and a patchy distribution of chemical data. Thus, in order to promote “chemosystematics” in Opiliones and to improve

the chemical data base for Laniatores, we here report on the chemistry of the scent gland secretion of *Pachylus paessleri* Roewer 1913 (Gonyleptidae: Pachylinae). This study adds comparative knowledge to an assemblage of pachyline genera (*Pachylus* C.L. Koch 1839, *Acanthopachylus* Roewer 1913, *Pachyloidellus* Müller 1918) that are deemed to be close relatives (Acosta 2002; Acosta unpubl. data). The chemistry of scent gland products of *Acanthopachylus aculeatus* (Roewer 1913) has already been analyzed (Estable et al. 1955; Eisner et al. 2004) and that of *Pachyloidellus goliath* Acosta 1993 by Acosta et al. (1993).

We received four adult specimens (three females, one male) of *Pachylus paessleri* from Pedro Avaria (Avaria Import & Export, Bochum, Germany) in August 2008. This harvestman species occurs in central Chile, where it is quite common, though no capture data were available to us. One male and one female are deposited as voucher specimens in the Arachnological Collection, Cátedra de Diversidad Animal I, Faculty of Exact, Physical and Natural Sciences, National University of Córdoba, Argentina (CDA), identified as Pp-0-A and Pp-0-B. We kept the specimens alive for about one year in a terrarium that contained pieces of wood, bark, leaves, litter and soil, and we fed them dead insects (mainly house crickets *Acheta domesticus* and mealworm larvae *Tenebrio molitor*). For extraction of scent gland secretions, individuals were squeezed moderately by hand, which immediately resulted in the discharge of large yellowish droplets from the ozopores. We collected these droplets on small pieces of filter paper (~ 2 × 3mm) and extracted the secretion-loaded filter papers (along with non-loaded pieces of filter paper as a blank) in small amounts of hexane (150 µl). After extraction (for about 15 min), we used an aliquot of the extract (usually 1 µl) for injection into our GC-MS system. We performed the analyses on a Trace gas chromatograph GC2000 coupled to a Voyager mass spectrometer (ThermoQuest, Vienna, Austria). The GC-column (a ZB-5MS fused silica capillary column: 30 m × 0.25 mm i.d., 0.25 µm film thickness from Phenomenex, Germany) was directly connected to the ion source of the MS. The splitless Grob injector was kept at 260° C, and we used helium 5.6 (at a constant flow rate of 1.5ml/min) as a carrier gas. All data in the text refer to the following

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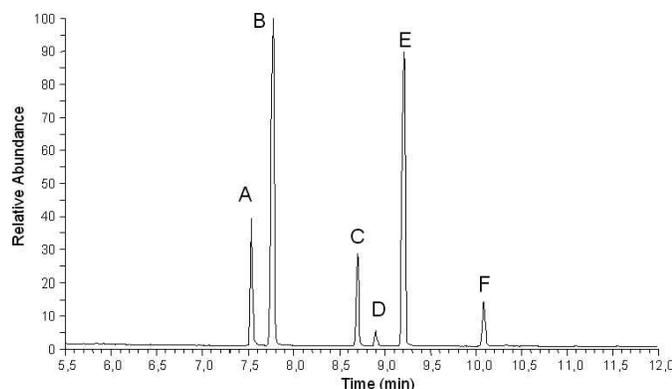


Figure 1.—Gas chromatographic profile of the scent gland secretion of a female of *Pachylus paessleri*. Each peak corresponds to a compound as follows: (A) 2,5-dimethyl-1,4-benzoquinone; (B) 2,3-dimethyl-1,4-benzoquinone; (C) 2-ethyl-3-methyl-1,4-benzoquinone; (D)\* 2-ethyl-5-methyl-1,4-benzoquinone; (E)\* 2,3,5-trimethyl-1,4-benzoquinone; (F)\* 2,5-dimethyl-3-ethyl-1,4-benzoquinone. Compounds marked with asterisk\* were tentatively identified on the basis of their mass spectra.

temperature program: Initial temperature 50° C for 1 min, followed by an increase of 10° C/min to 200° C, with 15° C/min to 300° C, and an isothermal hold for 5 min. The ion source of the mass spectrometer and the transfer line were kept at 170° C and 310° C, respectively. Electron impact (EI) spectra were recorded at 70eV. For reference, we used authentic 2,5-dimethyl-1,4-benzoquinone (Aldrich: Vienna, Austria). Quantification of compounds is based on the integration of peak areas in the chromatograms.

Secretions from both male and female *Pachylus paessleri* exhibited a consistent gas chromatographic pattern of six components in stable

relative amounts (peaks A–F in Fig. 1). All components showed the mass spectral characteristics of alkylated 1,4-benzoquinones. In order to differentiate between the substitution patterns in di- or even tri-substituted benzoquinones, we followed the basic fragmentation patterns described for alkylated benzoquinones (e.g. Budzikiewicz et al. 1964; Machado et al. 2005): 1) loss of carbon monoxide; 2) loss of the most substituted acetylene; and 3) fission into two halves, with elimination of the most substituted neutral fragment. The latter mechanism provides diagnostic information on alkyl substitution in benzoquinones: in the case of asymmetrically substituted benzoquinones such as for 2,3-alkylation, fission into two halves leads to a prominent ion at  $m/z$  54 (corresponding to the unsubstituted half of the molecule). By contrast, in case of alkyl substitution, other characteristic fragments are observed, corresponding to the fragment mentioned above, plus the mass of the respective alkyl-group (e.g., in symmetrically methylated benzoquinones, such as in 2,5-dimethylbenzoquinone, a characteristic ion at  $m/z$  68; i.e., 54 plus a  $\text{CH}_2$ -group, would be expected).

Compounds A and B, both showing a molecular ion at  $m/z$  136 (Table 1), appeared to be isomeric dimethyl-benzoquinones or ethyl-benzoquinones, respectively. Compound A exhibited an EI-fragmentation pattern with a base ion at  $m/z$  68 but no fragment at  $m/z$  54, indicating a 2,5-dimethyl-1,4-benzoquinone. A comparison to an authentic sample showed full correspondence of both spectral comparison and gas chromatographic retention time. For compound B (having no fragment at  $m/z$  68 in the spectrum) the structures of a 2-ethyl- or a 2,3-dimethyl-benzoquinone were possible. A comparison to the mass spectra of the authentic compounds (e.g., Machado et al. 2005) clearly supported the structure of a 2,3-dimethyl-1,4-benzoquinone.

All other components were tentatively identified on the basis of their mass spectra only (Table 1). Components C, D and E also appeared to be isomeric alkylated benzoquinones, all exhibiting molecular ions at  $m/z$  150. This is consistent with structures of 1)

Table 1.—Mass spectrometric identification of secretion components in *Pachylus paessleri*.

Peak	Retention time (min)	Relative abundance (% peak area)	Fragmentation pattern $m/z$ (relative intensity)	Identified structure	Formula
A	7.54	12	136 (79), 121 (3), 108 (37), 96 (24), 80 (21), 79 (46), 68 (100), 53 (8), 40 (36)	2,5-dimethyl-1,4-benzoquinone	
B	7.77	40	136 (100), 108 (69), 107 (76), 90 (16), 82 (69), 80 (32), 79 (85), 77 (18), 65 (11), 54 (87), 53 (45), 51 (27)	2,3-dimethyl-1,4-benzoquinone	
C	8.70	8	150 (100), 135 (11), 122 (36), 121 (19), 107 (93), 103 (7), 93 (10), 91 (10), 82 (37), 79 (61), 77 (29), 67 (19), 65 (16), 54 (41), 53 (28), 51 (14)	2-ethyl-3-methyl-1,4-benzoquinone	
D	8.90	1.5	150 (100), 135 (6), 122 (44), 121 (12), 107 (41), 103 (5), 96 (7), 93 (8), 91 (8), 82 (14), 79 (81), 77 (20), 68 (31), 67 (9), 54 (20), 53 (27), 51 (12), 40 (17)	2-ethyl-5-methyl-1,4-benzoquinone	
E	9.21	35	150 (100), 135 (3), 122 (75), 121 (42), 107 (93), 96 (30), 94 (23), 93 (26), 91 (17), 82 (17), 79 (91), 77 (36), 68 (93), 65 (10), 54 (53), 53 (50), 51 (30), 40 (36)	2,3,5-trimethyl-1,4-benzoquinone	
F	10.09	5	164 (95), 149 (15), 136 (28), 135 (14), 121 (100), 96 (9), 93 (39), 91 (22), 77 (20), 68 (25), 67 (22), 65 (12), 53 (18), 40 (17)	2,5-dimethyl-3-ethyl-1,4-benzoquinone	

ethyl-methyl-, 2) propyl-, and 3) trimethyl-benzoquinones, respectively. Comparisons of mass spectral data to spectra from the NIST-library did not lead to unequivocal results. Only compound C lacked a fragment at  $m/z$  68, indicating alkyl-substitution at only one side of the ring; i.e., 2,3-substitution. This situation left only the possibility of 2-ethyl-3-methyl-1,4-benzoquinone open. In addition, the EI-mass spectrum of compound C completely matched the spectrum of 2-ethyl-3-methyl-1,4-benzoquinone as given in Machado et al. (2005). By contrast, both compounds D and E exhibited fragments at  $m/z$  68 and were nearly indistinguishable by their EI mass spectra. Regarding the substitution patterns found in the already identified dimethyl-benzoquinones of *P. paessleri* (compounds A and B), the structures of 2-ethyl-5-methyl-1,4-benzoquinone and 2,3,5-trimethyl-1,4-benzoquinone appeared to be most likely. Since the latter compound is rather common and widely distributed in Gonyleptidae (see Gnaspini & Hara 2007), we tentatively propose the more abundant component E (about 35% of the secretion) to be 2,3,5-trimethyl-1,4-benzoquinone. Consequently, component D (a minor component) is proposed to be 2-ethyl-5-methyl-1,4-benzoquinone. The remaining component F had a molecular ion at  $m/z$  164, indicating a further analogous benzoquinone, which contained an additional  $\text{CH}_2$ -moiety, thus a  $(\text{C}_4\text{H}_{12})$ -1,4-benzoquinone. The definitive substitution pattern was not further analysed, but - considering the components detected so far - it probably is a dimethyl-ethyl-1,4-benzoquinone. Along with a fragment ion at  $m/z$  68 (indicating only one methyl-group on each side of the ring), the structure of 2,5-dimethyl-3-ethyl-1,4-benzoquinone is favored.

The two main components of *P. paessleri*, 2,3-dimethyl-1,4-benzoquinone (peak B) and 2,3,5-trimethyl-1,4-benzoquinone (peak E) together amounted for  $\frac{3}{4}$  of the secretion. Both compounds are not only shared with its presumed relatives, *Pachyloidellus goliath* and *Acanthopachylus aculeatus*, but proved to be widespread in Gonyleptidae (Hara et al. 2005). They are present in at least four other separate lineages within this family (i.e., Goniosomatinae, Gonyleptinae, Sodreaninae and "Pachylinae 4" sensu Hara et al. 2005), though not detected in the clade containing the Progonyleptoidellinae and in some Goniosomatinae. The two compounds also appear to be distributed among other families of Gonyleptoidea investigated so far, both of them in the family Cosmetidae and 2,3-dimethyl-1,4-benzoquinone also in the Manaosbiidae (Eisner et al. 1971, 1977; Roach et al. 1980). The remaining methyl-benzoquinone of *P. paessleri* (2,5-dimethyl-1,4-benzoquinone = peak A: about 12% of the secretion) is also known from the closely related *Acanthopachylus aculeatus* (Estable et al. 1955; Eisner et al. 2004), and from *Paecilaema eutypa* (Chamberlin 1925), a cosmetid species (Eisner et al. 1977), but was not found in *P. goliath* nor elsewhere in the Gonyleptidae. On the other hand, ethyl-benzoquinones appear to be less widespread in laniatorean secretions. However, at least 2-ethyl-3-methyl-1,4-benzoquinone (component C of our study) has recently been identified in *Acutisoma longipes* Roewer 1913, a goniosomatine gonyleptid (Machado et al. 2005, there referred to genus *Goniosoma*). The latter compound, as well as 2-ethyl-5-methyl-1,4-benzoquinone (component D of *P. paessleri*), may also occur in several other gonyleptids of different subfamilies, but an exact identification of the specific isomers is so far missing in the literature (Hara et al. 2005). Thus, 2-ethyl-5-methyl-1,4-benzoquinone (component D) and 2,5-dimethyl-3-ethyl-1,4-benzoquinone (component F) may be new for laniatorean (and also opilionid) secretions. However, the dimethyl-ethyl-benzoquinone may already have been found in another pachyline species, *Pachyloidellus goliath*, but has been classified there as another isomer (as 2,3-dimethyl-3-ethyl-1,4-benzoquinone) (Acosta et al. 1993). Thus, opilionid benzoquinones appear to be rather derived characters in Laniatores, since they appear to be restricted to gonyleptoids. They have been found in the three families of Gonyleptoidea so far investigated (Gonyleptidae, Cosmetidae, Manaosbiidae), and they are especially predominant in subfamilies of the Gonyleptidae. Consequently, the two widespread methyl-benzoquinones (our peaks B and

E) are probably symplesiomorphic on the taxonomic level of gonyleptoid families.

Regarding the plesiomorphic status of benzoquinones at the level of Pachylinae, not a single compound has been found to support the alleged relationship *Pachylus* + *Acanthopachylus* + *Pachyloidellus*. Nonetheless, in terms of similarity, the scent gland secretion profiles of *P. paessleri* and *A. aculeatus* look more similar to each other than to the secretion profile of *P. goliath*. This view is based on 1) 2,5-dimethyl-1,4-benzoquinone shared in secretions of *P. paessleri* and *A. aculeatus* while absent in other gonyleptids, including *P. goliath*, and 2) the lack of phenolic compounds (which represent a second chemical class in the secretion of *P. goliath*). These chemical affinities may be seen in accordance with the closer exomorphological similarities of *Pachylus* and *Acanthopachylus* (L.E. Acosta, pers. obs.). Also, the latter issue (i.e., presumed synapomorphic reduction of phenols) may provide a phylogenetic signal. Phenols may represent secretion components in laniatoreans that are phylogenetically older than benzoquinones. They are already present in the Phalangodoidea (rather basal Grassatores), as recently evidenced by the first investigation into the chemistry of the family Phalangodidae (Shear et al. 2010a), and 2-methyl-5-ethyl-phenol seems to represent an early compound (G. Raspotnig, unpubl. data). This and other phenols appear to be widespread in the secretions of Grassatores (e.g., Duffield et al. 1981), and are also still present in many Gonyleptoidea (e.g., some Cosmetidae, some Stygnopsidae, some Gonyleptidae), but presumably reduced in others (e.g., Eisner et al. 1977; Roach et al. 1980; Machado & Pomini 2008; Shear et al. 2010b). Disregarding the possibility of multiple convergent evolution, the presence of phenols in the secretion of *P. goliath* (Acosta et al. 1993) might thus be considered plesiomorphic. On the other hand, *P. paessleri* and *P. goliath* share ethyl-benzoquinones that might represent more derivative benzoquinone - characters of certain Gonyleptidae - this would relate *Pachylus* + *Pachyloidellus*, thus conflicting with the hypothesized clade *Pachylus* + *Acanthopachylus*. However, an ethyl-benzoquinone (homoplasy?) was also found in *Acutisoma longipes* (Machado et al. 2005), which is a representative of a clearly unrelated lineage, the Goniosomatinae.

This discussion shows how provisional our conclusions are at present. To develop the full potential of chemosystematics in the Opiliones, the generation of a reliable, comprehensive database is of uppermost importance. Not only more and more species (and lineages) should be incorporated into the chemical analyses, but compounds already reported might need confirmation and/or more analytical detail using modern techniques.

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